Proceedings of the Iowa Academy of Science

Volume 63 | Annual Issue Article 41

1956

Growth and Other Physiological Responses to Diethylstilbestrol in Diet of Rats and Guinea Pigs

Rodney Preston
Iowa State College

Edmund Cheng
Iowa State College

Wise Burroughs
Iowa State College

Growth and Other Physiological Responses to Diethylstilbestrol in Diet of Rats and Guinea Pigs*

By Rodney Preston, Edmund Cheng and Wise Burroughs

Recent studies have shown that the inclusion of diethylstilbestrol (stilbestrol) in rations of fattening beef cattle (1) and lambs (2) results in stimulation of liveweight gains and improvement of overall feed utilization in these species of animals. The physiological explanations for this action of stilbestrol are not apparent at this time since no such stimulation from stilbestrol has been noted in other species of animals. In studying the mode of action of stilbestrol in fattening beef cattle and lambs, it seemed desirable to first study the growth responses of certain laboratory animals (rats and guinea pigs) to see if they might be used as pilot animals in making detailed physiological measurements which might later be applied to cattle and sheep physiology. Previous work with rats has shown a growth inhibition with estrogens (3, 4); however, the levels administered have been relatively high.

Methods

Male rats of the Sprague-Dawley strain weighing initially 47-63 g. each were individually *ad libitum* fed for 5 weeks diets containing stilbestrol additions as follows: none, 0.01, 0.10, 1.0, 10.0 and 100.0 μg per g of diet. The diets fed were believed to be nutritionally adequate for good growth and consisted of ground corn 66.0, corn oil 4.0, dried skim milk 21.5, dried brewers yeast 4.0, wheat bran 1.0, cod liver oil 1.0, calcium bicarbonate 0.4, dicalcium phosphate 1.5, iodized salt 0.5, and trace mineral premix 0.1%. The stilbestrol additions were made by dissolving crystalline stilbestrol in ethanol, mixing the ethanol solution with the ration and finally removing the ethanol by warming on a steam plate.

The design of the experiment consisted of randomly allotting 8 rats to each ration and randomly assigning the position of cages to various levels of stilbestrol feeding. The room temperature was maintained between 70° and 74°F. The cages and feeders were so constructed that feed wastage was reduced to a minimum. The rats were weighed weekly and feed consumption recorded daily. Feeding was terminated at the end of 5 weeks at which time the animals were sacrificed and testicle weights recorded.

Male guinea pigs weighing from 275-347 g from the Gopher

*Journal paper No. J-2924 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 869

State Caviary were fed diets containing the following amounts of stilbestrol: none, 0.001, 0.01, 0.10, 1.0, and 10.0 μg per g of diet. The diet fed consisted of a commercially pelleted ration (Purina) for rabbits. The pellets were ground, the stilbestrol added in an ethanol solution, and following ethanol removal by drying, the diet was repelleted for purposes of reducing feed wastage. Ascorbic acid was given orally twice a week with a pipette to supply the daily requirement for this vitamin.

A randomized block design using initial body weights as outcome groups was used in assigning 4 guinea pigs to each ration treatment. Daily individual feed records and weekly body weights were recorded over a 7-week period. Blood serum inorganic phosphorus was determined using the Fiske and Subbarow (5) method. Blood serum phosphatase was determined by a modified Bodansky procedure (5) and blood serum glucose was analyzed using the Somogyi-Shaffer-Hartmann method (5). At the termination of the feeding period, the guinea pigs were sacrificed and the following organs dissected and weighed: anterior pituitary, thyroids, adrenals, seminal vesicles, testicles, liver, and kidneys. Estimations were also made of mammary development taking place with respect to the amount of stilbestrol fed.

Results

The growth results and feed efficiency responses obtained with the rats are presented in Table I. The lowest level of stilbestrol per g of diet (0.01 μg) failed to influence either body weight gain or feed required per unit gain. However, higher levels of stilbestrol significantly reduced body weight gains and feed efficiency. Alopecia also began to appear at about 3 weeks time on the backs of rats receiving 100 μg of stilbestrol per g of diet. This condition also occurred in about half of the animals receiving the 10 μg per g level.

Table I

Growth and Feed Efficiency Responses in Rats to Oral Administration of Stilbestrol, 8 Rats per Series

	μg. of stilbestrol per g of diet					
	0.0	0.01	0.10	1.0	10.0	100
Av 5-wk body wt gains (g)**	222	216	146	104	77	71
Av total feed consumed in 5-wk (g)**	607	587	461	374	320	316
Feed/g gain (g)	2.7	2.7	3.2	3.6	4.2	4.4

**Linear and quadratic responses of these curves were significant ($P<.01$)

Since Meites (6) found that injections of vitamin B_{12} helped to counteract growth inhibition resulting from large doses of stilbestrol fed to rats, half of the animals on each level of stilbestrol were in-

Table II

Growth and Feed Efficiency Responses in Guinea Pigs to Oral Administration of Stilbestrol, 4 Guinea Pigs per Series

	μg stilbestrol/g of diet					
	0.0	0.001	0.01	0.10	1.0	10.0
Av 7-wk body wt gain (g)**	299	328	372	318	220	128
Av total feed consumed in 7-wk (g)**	1807	1983	2104	1838	1591	1317
Feed/g gain (g)	6.0	6.0	5.7	5.8	7.2	10.3

**Linear and quadratic responses of these curves were significant ($P<.01$).

jected with a mixture of 11 B-vitamins at a rate estimated to supply 10 times the daily requirement during the last 2 weeks of the feeding period. This treatment did not alter the response to stilbestrol. Testicle weights of rats receiving increasing amounts of stilbestrol were correspondingly reduced in size suggesting atrophy or inhibition in development.

The liveweight gains and feed required per unit of gain in the guinea pigs are presented in Table II.

Table III

Effect of Oral Administration of Stilbestrol on Average Organ Weights and Serum Constituents of Guinea Pigs

	μg of stilbestrol/g of diet						
	0.0	0.001	0.01	0.10	1.0	10.0	Standard Error[1]
Serum constituent:							
Inorg. Phosphorus (mg%)	8.1	7.4	8.4	7.9	6.5	6.5	0.4
Alkaline Phosphatase[2]	8.6	7.8	6.3	6.9	4.2	2.2	0.6
Glucose (mg%)	166	150	164	155	142	141	10.5
Organ:[3]							
Ant. pituitary (mg)	7.9	8.5	8.0	7.7	11.3	8.6	1.0
Thyroids (mg)	95.9	104.9	98.2	92.8	68.9	59.6	8.9
Adrenals (mg)	268	250	320	325	276	370	29.4
Seminal Vesicles (g)	2.2	2.7	3.3	2.9	1.3	0.4	0.2
Testicles (g)	3.2	3.5	3.7	3.8	2.6	0.6	0.2
Liver (g)	22.1	23.2	27.8	24.1	20.1	18.8	1.2
Kidneys (g)	4.5	4.6	4.9	4.8	4.2	4.1	0.2
Mammary[4]	0.3	0.5	1.3	3.5	8.3	7.3	....

[1]Standard error of each treatment mean

[2]Bodansky units

[3]All organ weights were fresh weights except anterior pituitary glands, which were fixed in Bouin's fluid before weighing.

[4]Visual estimation: 0 — no stimulation, 10 — maximum stimulation observed.

It can be seen that when stilbestrol was present in the guinea pig diets at levels of 0.001 and 0.01 μg per g of diet, there was an apparent stimulation in body weights gains. Above these levels, body weight gains steadily decreased. Also feed requirements per unit of body weight gain tended to decrease with the lower levels of stilbestrol feeding and to increase with the higher levels of feeding.

The results obtained in the blood studies and organ weights of the guinea pigs are presented in Table III. It can be seen that the presence of stilbestrol in the diet reduced serum alkaline phosphatase activity. Little influence was noted on blood inorganic phosphorus or serum blood glucose. Some of the organs, notably the thyroids, adrenals, seminal vesicles, testicles, and mammary gland appeared to be influenced by stilbestrol feeding.

Discussion

The results obtained in this investigation when stilbestrol was orally administered to growing male rats are in general agreement with previous work (3, 4) where larger amounts of stilbestrol were fed. It appears that the growth of the rat is inhibited when levels of stilbestrol higher than 0.01 μg per g of diet are administered. In this study, the lowest average total daily intake of stilbestrol was 0.17 μg per rat daily. Body weight gains at this low level were almost identical to the controls. Certainly there was no stimulation at this level. The growth inhibition of rats on higher levels of stilbestrol intake can best be explained on the basis of a stress mechanism resulting in the stimulation of the adrenal gland via the anterior pituitary. Various hormones from the adrenal gland have been shown to be inhibitory to the growth of rats (7).

The results with guinea pigs suggest that the growth rate of growing male guinea pigs can be increased by the incorporation of stilbestrol into the diet at low levels. At higher levels, however, growth rates are reduced similar to results obtained with rats. Two comparisons can be made between guinea pigs and beef cattle and lambs which may have some bearing upon their common growth response to stilbestrol feeding. First, guinea pigs are roughage consumers. Brooks, *et al* (8) have shown that stilbestrol stimulated cellulose digestion by rumen microorganisms removed from sheep. Rabbits, also roughage consumers, may be stimulated by the injection of estrogens (9). Secondly, stilbestrol may have some influence upon the production or metabolism of fatty acids produced in the digestive tract as the result of microbial fermentation, since both ruminants and guinea pigs produce relatively large amounts of these acids (10,11), as compared to rats and other animals subsisting on low-fiber diets.

Summary

Oral administration of stilbestrol at either low or high levels reduced growth rate of male rats. With growing male guinea pigs, however, body weight gains were stimulated with very low levels of stilbestrol, but were reduced with higher levels. Serum alkaline phosphatase in guinea pigs was reduced by stilbestrol administration. Also various organs dissected from guinea pigs were influenced by stilbestrol feeding. In many respects the response of the guinea pigs to oral administration of stilbestrol was more nearly like that noted in ruminants as contrasted to the dissimilar behavior noted in rats and other animals subsisting on low-fiber diets.

Literature Cited

1. Burroughs, W.. Culbertson, C. C., Cheng, E., Hale, W. H. and Homeyer, P., J. Animal Sci., 1955, v 14, 1015.
2. Hale, W. H., Homeyer, P. G., Culbertson, C. C. and Burroughs, W., J. Animal Sci., 1955 v 14, 909.
3. Emery, F. E., Growth, 1943, v 7, 227.
4. Noble, R. L., J. Endocrinology, 1939, v 1, 128.
5. Hawk, P. B., Oser, B. L. and Summerson, W. H., *Practical Physiological Chemistry,* 12 ed, The Blakiston Co., New York (1947).
6. Meites, J., Iowa State College J. Sci., 1953, v 28, 19.
7. Parmer, L. G., Katonah, F. and Angrist, A. A., Proc. Soc. Exp. Biol. Med., 1951, v 77, 215.
8. Brooks, C. C., Garner, G. B., Muhrer, M. E. and Pfander, W. H., J. Animal Sci., 1953, v 12, 909 (Abs.).
9. Campbell, I. L. and Turner, C. W., Missouri Agri. Exp. Sta. Res. Bull. 352, 1942.
10. McClymont, G. L. Australian J. Sci. Res., 1952, v 5, 374.
11. Hagen, P. and Robinson, K. W., Australian J. Exp. Biol. Med. Sci., 1953, v 31, 99.

Iowa Experiment Station
Ames, Iowa